THE EFFECT OF KENO™PURE AND KENOSTART™ TEAT DISINFECTANTS AS PART OF PREVENTION AND CONTROL OF BOVINE MASTITIS IN HOLETA RESEARCH CENTER DAIRY FARM, CENTRAL ETHIOPIA

M.Sc. Thesis

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APPROVAL SHEET

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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the Council of Graduate Studies (CGS) through the Departmental Graduate Committee (DGC) of the candidate's major department.

DEDICATION

I dedicate this manuscript to my father Ato Afera Merete and my mother W/o Wesene Wodajio for their consistent and unconditional support throughout my educational carriers.

STATEMENT OF AUTHOR

I declare that this thesis is my own work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for M.Sc. Degree, to Jimma University College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author, Yeshihareg Afera was born on July 26, 1986 G.C in Lume wereda, Oromia Regional State, East Shewa Zone, Ethiopia. The author attended her elementary education at Ejersa Elementary School from 1993 to 1999, and then she started her junior secondary education at Modjo Junior Secondary School in 2001. She continued her secondary school at Bishoftu Compressive Secondary School in Bishoftu and completed her preparatory education in 2003. Then, she joined Jimma University College of Agriculture and Veterinary Medicine in 2004, and graduated with a Degree, Doctor of Veterinary Medicine (DVM) in Veterinary Medicine in 2008. Soon after her graduation, she was employed by Oromia Regional State in Jimma Zone Dedo Wereda as Veterinary Doctor and served for six months. By the next year, she was joined Hirna Regional Veterinary Laboratory of West Hararghe and served for one year. She again joined Jimma University College of Agriculture and Veterinary Medicine in March 2010 to pursue her M.Sc. study in Veterinary Epidemiology.

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LIST OF ABBREVIATIONS

ASC	Acidified sodium chlorite
BMSCC	Bulk milk somatic cell count
CI	Confidence interval
CM	Clinical mastitis
CMT	California mastitis test
CNS	Coagulase-negative staphylococci
CSA	Central Statistical Agency
DNA	Deoxyribonucleic acid
ETB	Ethiopian birr
€	Euro
JUCAVM	Jimma University, Collage of Agriculture and Veterinary Medicine
KCBS	Kosovo cluster and business support
LDBSA	Linear benzene sulfonic acid
NMC	National Mastitis Council
PMN	Polymorpho-nuclear cells
Rs.	Rupee
SPSS	Statistical Package for Social Sciences
WBC	White blood cells

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ABSTRACT

Mastitis is one of the most important and expensive disease of dairy industry resulted in severe economic losses from reduced milk production, treatment cost, increased labour, milk withheld following treatment and premature culling. Prevention and Control of bovine mastitis are thus essential for the production of high quality milk. An experimental trial was conducted in Holeta research center dairy farm from June to December 2011 to assess the effect of KENO™PURE and KENOSTART™ teat disinfectants on reducing the incidence of mastitis, improving teat-skin and teat-end conditions and to estimate and compare the direct financial loss due to clinical mastitis cases between treatment and control groups using randomized controlled trial method. The incidence of subclinical mastitis was diagnosed by using California mastitis test. Out of a total of 38 lactating dairy cows that were randomly allocated into treatment and control groups;19 cows were dipped with KENO™PURE solution and KENOSTART™ teat dip before and after milking respectively whereas the rest 19 cows were left untreated. Teat end and teat skin condition of each study cows were scored every 20 days. The direct financial loss due to treatment of clinical mastitis cases in treated and control groups was computed. Accordingly, a greater number of new intramammary infections were found in the control group than in the treatment group. One hundred fourteen (22%) quarters in the control group and 42 (8%) quarters in the treatment group were positive for new intramammary infection (χ^2 = 41.592, P <0.05). The incidence rate at cow level shows a significant variation (P <0.05, χ^2 = 31.222) between treated 23(17.3%) and control 65 (49.6%) groups respectively. The incidence of clinical mastitis occurred at quarter level in dipped cows was 2 (0.4%) as compared to non-dipped ones which was 7 (1.4%), (χ^2 = 2.875, p> 0.05).The treatment groups had a significantly more smooth teat skin and smooth sphincter teat ends condition than the control group (P<0.05). The financial loss of treatment of clinical mastitis in dipped cows was minimal as compared to non-dipped group. In conclusion, the application of pre and post teat disinfectants has significantly decreased the incidence of subclinical mastitis and has improved the teat skin as well as the teat end condition and reduced the direct financial loss due to treatment of clinical mastitis. Hence, teat dips have to be introduced in to the dairy farms as part of the mastitis prevention and control programs.

Key words: *Dairy Cows, Holeta, KENO™PURE, KENOSTART™, Mastitis*

1. INTRODUCTION

Ethiopia's estimated livestock population is often said to be the largest in Africa, at approximately 150 million head (CSA, 2009/2010). The recent census estimates about 52 million cattle, 33 million sheep, 30 million goats, 1.9 million horses, 5.7 million donkeys and 400,000 mules. In addition, there are approximately 2.5 million camels, and 42 million poultries. Animals make an important contribution to livelihoods in smallholder farming systems throughout the developing world (Powell *et al.,* 1998). Like in many developing countries, domestic animals play a crucial role in Ethiopia. They constitute a source for traction power, income, a provision of milk and meat (Mekonnen *et al*., 1989).

Despite the huge wealth, milk production often does not satisfy the country's requirements due to a multitude of factors. Among the various factors, disease of the mammary glands known as mastitis has a great role for reduction of milk production (Fekadu, 1995). Mastitis is the most important and expensive disease of dairy industry resulted in severe economic losses from reduced milk production, treatment cost, increased labour, milk withheld following treatment and premature culling (Miller *et al.,* 1993). Globally, the losses due to mastitis amount to about 35 billion dollars annually (Ratafia, 1987). Mastitis in both clinical and subclinical forms is a frustrating, costly and extremely complex disease that results in a marked reduction in the quality and quantity of milk (Harmon, 1994).

Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for breeders and consequently influences the national income of many countries (Ramachandrainh *et al*., 1990). Depending on differences in management practices and animal care, the impact of the disease varies between herds. Some farmers detect mastitis in their dairy cow quite often, while others rarely treat their cows for clinical mastitis (Barkema *et al*., 1998). Mastitis is a difficult disease to control because many different bacteria are capable of infecting the udder and producing the disease.

Control of mastitis in dairy cows is thus essential for the production of high quality milk. Moreover, prevention of bovine mastitis is the most important component of a mastitis control program and both pre and post milking teat antiseptics are the most effective procedures for

preventing new intramammary infection in dairy cows. These procedures involve dipping teats of dairy cows before and after milking with an appropriate germicidal preparation to reduce teat skin colonization and contamination with mastitis-causing bacteria and minimize penetration into the teat canal (Nickerson, 2001).

Teat dips, both pre-milking and post-milking, are widely used topical antimicrobial formulations to kill bacteria which cause mastitis. Antimicrobials used in teats include iodine (most common), chlorhexidine, surfactants (such as linear dodecyl benzene sulfonic acid), organic acids, quaternary ammonium salts, and acidified sodium chlorite (ASC) (Cayce and Kere, 2002**)**.

Iodine is widely used as an active ingredient in the majority of mastitis control dips, with concentrations ranging from 0.10 to 1.0 % (Leslie *et al*., 2005). Iodine teat dips are effective at reducing the spread of bovine mastitis, and free iodine is an important factor in determining the germicidal efficacy of these teat dips (Gottardi, 1991).A natural exposure field trial showed that a dip containing 12 to 16 ppm of free iodine provided greater reduction of new infections (Foret *et al*., 2005).

KENOSTART™ is a premium conditioning post dip which is based upon stable iodine: has a skin neutral pH (non-irritating) and is a non-dripping dip due to its high viscosity. It allows total security around the teat and demonstrated to be efficient against bacteria causing mastitis. It is tested according to European Standards EN 1656 (field conditions) against *Staphylococcus aureus, Escherichia coli, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Corynebacterium bovis,* and registered as an aid in mastitis prevention program (CID LINES, 2007).

KENO™PURE is a new drug which contains lactic acid as an active pharmaceutical ingredient and applied during pre-milking period. It allows a complete preparation of the teats before milking and also encourages the release of oxytocin that stimulates milk let-down. It can be applied with a foaming dip cup, a sprayer or add in a bucket of water for cloth disinfection (CID LINES, 2007).

Normally, teat dipping is a simple, effective, and economical means to reduce bacterial populations on teat skin both before and after milking. A plenty of published evidence shows that this practice reduced the rate of infection among dairy cows. For example, pre-dipping is found to reduce the incidence of new intramammary infections with environmental pathogens by greater than 50% compared with udder washing and drying with individual paper towels (Nickerson, 2001). In addition to pre-dipping, the vast majority of post milking teat dip products reduced the new infection rate by at least 50% and some products even as high as 95% and they are now widely accepted (Nickerson, 2001).

Generally the importance of teat dipping practice/disinfectant in preventing and controlling of mastitis is proved to be effective in other parts of the world. However, no investigation was done so far regarding its effectiveness in Ethiopia. In addition, dips that have recently appeared on the market are lacking research/baseline data. Therefore, experimenting of teat disinfectant is a necessary first step to ensure its effectiveness before introducing for utilization, so the aim of this study is to assess the effect of KENO™PURE and KENOSTART™ teat disinfectants on reducing the incidence of mastitis, improving teat-skin and teat-end conditions and to estimate and compare the direct financial loss due to treatment of clinical mastitis cases between the study groups.

2. LITERATURE REVIEW

2.1. Definition

Mastitis is an inflammation of the mammary gland which can be caused by physical or chemical agents but the majority of cases are infectious and usually caused by bacteria (Quinn *et al.,* 2004). These bacteria have penetrated and enter the udder through the teat ends except specific germs such as mycoplasma (Ruben and David, 2003). Therefore, bacteria found in the udder do not go from quarter to quarter without going out of the opening of one teat and into the opening of another (Schroeder, 2010).

2.2. Mastitis Causing Bacteria

The most common causes of udder disease include staphylococci (*Staphylococcus aureus* & *Staphylococcus epidermidis*), streptococci (*Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis & Streptococcus bovis*) and coliforms (mainly Escherichia coli & Klebsiella pneumoniae). Other less frequent agents include Pseudomonas, Nocardia, Mycoplasma and yeast (Khan and Khan, 2006). Coagulase negative staphylococcus (CNS) is also the prevalent bacterial pathogen in udder infections (Lafi *et al.,* 1994). Mastitis causing pathogens can also be categorized into contagious and environmental pathogens. The most common contagious pathogens are *Staphylococcus aureus* and *Streptococcus agalactiae*. These spread from infected to clean udders during the milking process through contaminated milker's hand and cloth towels used to wash or dry udder of more than one animal and may be by flies. Contagious organisms are responsible for most of clinical cases and *Staphylococcus aureus* is at the top of the list in dairy species of animals (Allore, 1993). Among environmental pathogens, the most common bacteria are *Streptococcus uberis, Streptococcus dysgalactiae,* coliforms such as *Escherichia coli* and Klebsiella. Transmission of the environmental pathogens occurs between milking. Coliform infections are usually associated with unsanitary environment, while Klebsiella are found in sawdust that contains bark or soil. Coliform infections manifest symptoms of abnormal milk, swollen udder/quarters, watery milk and depressed appetite or elevated body temperature (Bramley, 1997).

2.3 Development of the Disease

Mastitis begins after bacteria pass through the teat duct and enter the cisternal area. Invasion of the teat usually occurs during milking. Organisms present in the milking machine or at the teat end enter the teat canal and cistern when there is admission of undesired air in the milking unit (Mustafa, 2003). Usually, after milking, the teat canal remains dilated for 1-2 hours while the canal of a damaged teat may remain partially open permanently. This makes it easier for organisms from the environment or those found on injured skin to enter the teat canal (Nickerson *et al*., 1996; Neijenhuis *et al*., 2000).

Adherence of bacteria to tissues lining cisterns and ducts may prevent flushing-out during milking and help establish infections. Bacteria eventually enter the glandular tissues where they affect alveolar cells. Toxins produced by bacteria cause death of or damage to milk-secreting epithelial cells, and these cells produce substances to the blood stream that increase blood vessel permeability. This allows leukocytes to move from the blood into the alveolus where they function by engulfing bacteria (Mustafa, 2003).

Infectious lesions of teat skin and teat end

Infectious lesions of teat skin are usually caused by viruses, pyogenic bacteria, necrotizing bacteria or fungi. Viral infections usually cause primary lesions. Bacterial infections may cause primary lesions or be secondary infections of pre-existing viral lesions or trauma. The initial trauma may be milking-machine induced damage or be caused by any of the environmental factors (Hillerton *et al.,* 2001).

Pseudocowpox is a disease caused by *Pseudocowpox virus*, (also known as "Paravaccinia") a virus of the family *Poxviridae* and the genus *Parapoxvirus* (James and Berger, 2006). Pseudocowpox, a paravaccinia virus causes acute infection in young cows after calving or cows introduced to a herd that has the virus infection. Spread of infection can be relatively slow. Immunity is short-lived, lasting four to six months, and infections can be a chronic problem in some herds. As a consequence, cows in affected herds are likely to suffer repeated infections (Ohnstad *et al.,* 2007).

Lesions begin as small, red papules on the teats or udder. These may be followed rapidly by scabbing, or small vesicles or pustules may develop before scabs form. Scabs may be abundant but can be removed without causing pain. Granulation occurs beneath the scabs, resulting in a raised lesion that heals from the center and leaves a characteristic 'horseshoe' or circular ring of small scabs. This stage is reached in ~7-12 days. Some lesions persist for several months, giving the affected teats a rough feel and appearance, and more scabs may form. The infection spreads slowly throughout milking herds (Aiello, 1998). Milkers may develop localized lesions usually on their hands, i.e. 'milkers' nodules'. No specific treatment exists. Spread of infection can be minimized by milking infected cattle at the end of the run and wearing gloves (Ohnstad *et al.,* 2007).

Teat warts

Several strains of bovine papillomavirus cause the development of papillomas or fibropapillomas on teats including the 'rice grain' flat white warts (strain BVP-5), frond-like papillomas that protrude in a ragged fringe of up to one centimetre in length (strain BVP-6) and fibropapillomas that protrude from the teat surface (strain BVP-l) (Ohnstad *et al.*,2007).

In some herds, pale, smooth, raised lesions develop frequently on teat skin and may persist indefinitely without causing problems. In other instances, filamentous or frond-like lesions develop at the teat orifice and interfere with milking. Bovine warts are spread by direct or indirect contact (Aiello, 1998).

Teat-end hyperkeratosis

Hyperkeratosis it is a normal physiological response to the forces applied to the teat skin during milking, either by a milking machine, a hand-milker or a calf (Ohnstad *et al.,* 2003).The onset and severity of hyperkeratosis is profoundly influenced by the over-riding effects of climate, seasonal and environmental conditions, herd milk production level and genetics of individual cows (Ohnstad *et al.,* 2003). Teat-end hyperkeratosis is a thickening of the skin that lines the teat canal and surrounds the external teat orifice. The condition is variously described as teat rings, teat flowers, teat erosion, callus formation, callosity, cornification or teat-end roughness. Histological examination of teat sections confirms that

teat-end hyperkeratosis results from a localised hyperplasia of the *Stratum corneum* (Neijenhuis *et al*., 2001) and *Stratum granulosum* skin layers (Hamann *et al*., 1994).

Black spot

This is a secondary bacterial infection around the teat orifice by *Fusobacterium necrophorus* alone or in association with *Staphyloccocus aureus*. Blackspot may be superimposed on either moderate or severe hyperkeratosis. The teat end appears ulcerated and blackened with granulation tissue present (Jackson and Cockcroft, 2002).

Teat injuries

Teat canal is the main route of entry of mastitis causing organisms except tuberculosis mastitis (haematogenous route), hence, teat injury is most important risk factor of intramammary infection. Changes to teat tissue, particularly the skin of the barrel, teat-end and teat canal may favour penetration of bacteria into the udder and increase the risk of new mastitis infections (Hamann *et al*., 1994).

Insect damage

Insect damage to teats may be caused by blood-sucking flies (most commonly mosquitoes, midges, sand-flies, black flies or biting flies), nuisance flies or wasps. The cause is usually easy to observe and pin prick wounds or bites, often with an inflammatory reaction, are obvious on the teat orifice or barrel. Nuisance flies exacerbate primary damage by abrasion of wounds to create larger sores (Ohnstad *et al.*, 2007). Persistent fly worry may cause self-mutilation by licking and produce abrasions on the teats (Jackson and Cockcroft, 2002).

Generally various agents and mechanisms, causing a number of forms of trauma or lesions, may affect the condition of the teats of the milking dairy cow fall into three broad categories, thus Machine milking effects, Environmental effects and/or agents and Infectious agents; as indicated in Table 1 (Hillerton *et al.*,2001).

Table 1: Teat conditions observable according to the cause of the problem.

Machine induced	Environmental	Infectious
Discoloration	Chapping	Pseudocowpox
Oedema	Mud sores	Herpes mammillitis
Congestion	Congestion Suckling damage	Cowpox
Wedging	Insect damage	Papilloma
Ringing	Other abrasions and cuts	Foot and Mouth Disease
Hemorrhaging - petechia	Weather damage	Vesicular Stomatitis
Hemorrhaging - larger	Allergic reactions	Ringworm
[Hyperkeratosis]	Photosensitization	*Staphylococcus aureus*
	Chemical damage	*Streptococcus dysgalactiae*
		Arcanobacterium pyogenes
		Fusiformis necrophorum

Source: Adapted from Hillerton *et al.*, (2001).

Teat condition

The teat canal is the primary physical and chemical barrier to invasion of mastitis pathogens into the udder (Hamann, 1987). The smooth muscles surrounding the teat duct should be contracted and the teat canal tightly closed between milkings to impede bacterial passage from the teat orifice into the interior of the gland (Nickerson, 1994). A teat-end in good condition is an important resistance factor to bacterial colonization of the mammary gland (Neijenhuis *et al*., 2001). Changes in teat tissue by milking, teat canal integrity, and teat tissue pliability may favour penetration of bacteria into the udder (O'Shea *et al.*, 1987).

2.4. Diagnosis

2.4.1. Physical examination

The normal mammary gland of cows is bilaterally symmetrical and soft in texture. The supra-mammary lymph node in dairy cow is normally palpable high in the rear udder (Jackson and Cockcroft, 2002). Heat, pain and swelling, firmness of the quarter, supra mammary lymph node enlargement, irregularities of tissue texture , flakes, clots, and other changes in milk composition or colour change are indicators of clinical mastitis (Sudhan and Sharma, 2010).

2.4.2. California mastitis test (CMT)

The California Mastitis Test (CMT) remains the only reliable screening test for subclinical mastitis that can be easily used on farm level (Leslie, 2002). The CMT was developed to test milk from individual quarters but also been used on composite and bulk milk samples. It is a simple, test for determining the number of nucleated cells (both neutrophils and epithelial cells) in the milk. Equal quantities of milk and commercial CMT reagent, which contains 3% alkyl aryl sulfonate and bromocresol purple (as a pH level indicator), are mixed in the cup on a white paddle and swirled. The reaction consists of a modification of the viscosity of the solution. The more nucleated cells, the thicker become the solution. The thickness of the gel produced is often scored as negative, trace, 1+, 2+, or 3+ and the probability of mastitis enhances in a direct proportion (Thieres *et al.*, 1999; Xia, 2006).

Fresh unrefrigerated milk can be tested using the CMT for up to 12 hours. Reliable readings can be obtained from refrigerated milk for up to 36 hours. If stored milk is used, the milk must be thoroughly mixed prior to testing because somatic cells tend to segregate with milk fat. The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time (Table 2) (Sharma *et al.* 2009).

Table 2: CMT reaction graded by intensity of gel formation

CMT score	Description	Interpretation
N (Negative)	No change	Healthy quarter
T (Trace)	Slime formed which disappeared with continuous movement of paddle	Sub clinical mastitis
1 (Weak)	Distinct slime, but no gel formation	Sub clinical mastitis
2 (Distinct positive)	Viscous with gel formation, which adhered to the margin.	Serious mastitis infection
3 (Strong positive)	The gel formation with convex projection, the gel did not dislodge after swirling movement of the paddle	Serious mastitis infection

Source: Adapted from Sudhan and Sharma, 2010

2.4.3. Somatic cell count (SCC)

The presence of cells in bovine milk, so-called 'somatic cells', has been recognised and studied for many years. More than 95 percent of somatic cells are leucocytes, including neutrophils, macrophages and lymphocytes. The somatic cell count (SCC) is that the number of somatic cells per millilitre of milk (Bradley and Green, 2005). This cell count is the most widely used indicator for inflammation to assess udder health and milk quality on quarter, cow, and herd level (Rasmussen *et al.*, 2005; Hamann, 2005). An increased BTSCC is related to increased herd infection prevalence and decreased milk production. The normal SCC in milk is generally below 200,000 per millilitre, but may be below 100,000 in first lactation animals or in well-managed herds. SCC above 250,000 - 300,000 is considered abnormal and nearly always is an indication of bacterial infection causing inflammation of the udder (Rice and Bodman, 1993).

Once an inflammatory response has been initiated, PMNs are the first cells to be recruited to sites of infection. The recruitment of PMNs into infected mammary gland is a normal part of the cow's defence mechanism. It is very effective for eradicating the majority of infections that occur (Riollet *et al.*, 2000; De Haas, 2003). In mammary glands that are infected with mastitis-causing pathogens, milk somatic cells consist for >95% of PMNs and PMNs are thus indicators of inflammatory response (Pillai *et al.*, 2001; De Haas, 2003).

Quarter somatic cell count

An uninfected udder will typically have a SCC less than 100,000 cells/ml. When the SCC is 100,000 to 199,999 cells/ml, the presence of infection can only be ruled out by bacteriological testing (Smith *et al.*, 2001).

The practical use of SCC data to determine cow infection status requires the selection of a threshold level (Radostits *et al.*, 1994), however, stressed that somatic cell counts are general indicators of udder health which are subject to many factors including age, stage of lactation, season, stress and management. Mean SCC decreased markedly soon after the beginning of lactation and increased during late lactation. The basic patterns of change over lactation remain the same in health or mastitic cows (Auldist *et al.*, 1995). However, Harmon (1994)

argued that marked increases in SCC are a result of cells being attracted to the mammary tissue because of direct mediators produced during a local infection; events that do not affect udder health are unlikely to have a direct or dramatic effect on SCC. According to him, little evidence has existed other than normal variation and any factor did not have a major influence on SCC in the absence of intramammary infection.

To determine specificity and sensitivity of SCC (for quarters, cow or bulk milk) several studies have been conducted. Larsen (2000) reported sensitivities ranging from 73-89% with corresponding specificities of 75-85% using threshold of 200,000 cells per millilitre taking culture as "gold standard". Sensitivity and specificity is affected by threshold (cut point for intramammary infection). Emanuelson (1997) used a threshold of 200,000 cell/ml for cow level to monitor herd mastitis in Sweden. The threshold for milk quality has no relation to the definition of udder health categorization. A threshold of 100,000 cells/ml can be assumed an internationally accepted definition of udder health (Hamann, 2005). Less than 200,000 cells/ml for cow and less than 130,000 cells/ml for bulk tank milk were reported by Larsen (2000).

Cow somatic cell counts from milk-recording

Milk recording data are often used to assess performance and udder health on dairy farms. The SCC is given on cow-level. When udder health assessment is exclusively based on cow SCC, the extent of CM (treated and untreated cases) may be underestimated. Dry cow mastitis, effectively cured mastitis between two recordings and strategies like blinding of infected quarters may not be evident in the recordings as elevated cow SCC. The genetic correlation between SCC and CM based on Scandinavian data is on average 0.7 and suggests that SCC and CM are partly the expression of the same trait (Rupp and Boichard, 2003), despite a low phenotypic correlation (around 0.3). The authors assume that based on monthly SCC only 30% of CM would be detected, i.e. those cases of CM with longer duration of elevated SCC at cases of CM that occur close to the monthly milk recording (Rupp and Boichard, 2003).

Somatic cell count and infection status

The primary factor affecting SCCs is infection status. Historically, many other factors have been blamed for high SCCs and poor udder health. Scientific studies have investigated many of these factors such as lactation number, stage of lactation, estrus, exercise, heat stress, stray voltage and day-to-day variation may all be related to small SCC changes as describe by Laevens *et al.,* (1997) and Alex **(**2009). However, it is important to memorize that the relationship between these factors and SCC is not cause-and-effect. These factors may exert an effect on the SCC but only if the cow also has an intramammary infection. These factors will not have the same effect on an uninfected mammary gland. In other words, if an infection is present in the mammary gland and these other factors cause a drop in milk production, the SCC may be exaggerated because of a concentration effect (Harmon, 1994).

2.4.4. Bacteriological culturing

Bacteriological culturing can be executed at herd, as well as cow and quarter level, each with its own specific goal. Bacteriological culturing at quarter level is the most often used as a diagnostic tool to solve mastitis problems. Knowledge on the infectious status of mammary glands can also be very helpful to prevent transmission of pathogens by diagnosing a reservoir at an early stage (Jayarao *et al*., 2004).

Most mastitis control programs include the use of individual cow cultures to determine which mastitis pathogens are present on the farm. Culturing can be used in a targeted fashion for specific control programs such as segregation plans for contagious mastitis or for surveillance to detect the presence of new or emerging pathogen. Culturing is also used to evaluate treatment efficiency and to establish susceptibility patterns to aid in the development of rational treatment strategies (Larsen, 2000).

2.5. Treatment

The drug of choice in the treatment of mastitis is one to which the bacteria are sensitive and which achieves high concentrations in the mammary gland without provoking tissue changes (Quinn *et al.,*2004).The most valuable mastitis treatment will minimize the amount of

milk discarded while maximizing efficacy against pathogens (Sanchez, 2010).The target site may depend on the causative agent: streptococci are known to remain in the milk compartment, but *Staphylococcus aureus* penetrates udder tissue and causes deep infection (Erskine, 2003).

Treatment and control of mastitis techniques to be used in cow is supported by a laboratory culture and antibiotic sensitivity examination. Both udder infusion and systemic antibiotic administration in case of acute severe mastitis is recommended (Guss, 1992).

2.5.1. Intramammary treatment

The most common route of administration of antimicrobials in mastitis is the intramammary route (Gruet *et al.,* 2001). Intramammary administration permits delivery of the antibiotic directly to the mammary gland. However, intramammary preparations are used, often in conjunction with parenteral preparations (depending on severity of infection) in per-acute and acute mastitis. Intramammary antibiotics are distributed unevenly in an inflamed gland due to inflammation, swelling, and fibrosis that can block milk ducts, thereby preventing antibiotic diffusion throughout the gland (Owens and Nickerson, 1989; Owens and Nickerson, 1990).

In addition, the intracellular location of some bacteria such as *Staphylococcus aureus* means that estimates of *in vivo* milk phase concentrations may not accurately reflect intracellular concentrations, because antibiotics have variable penetration to the site of infection in mastitis (particularly in chronic infections), it is likely that the minimum inhibitory concentration values determined *in vitro* do not accurately reflect the in *vivo* response to therapy (Owens and Nickerson, 1989). It is evident that bovine mammary gland is in general a difficult target for antimicrobial treatment, which is reflected in the low response of intramammary treatments (Craven, 1987).

2.5.2. Systemic treatment

The systemic treatment would penetrate throughout the udder better and be more efficient than intramammary treatment in therapy of mastitis. Systemic treatment of mastitis is widely adopted in the Nordic countries and this practice still continues (Ekman *et al.,* 1994).

However, the superiority of systemic treatment of mastitis over intramammary treatment is never proven in comparative clinical trials.

Treatment of clinical mastitis

Successful treatment of clinical mastitis often requires a history of the herd, isolation, identification and susceptibility pattern of the bacteria involved and relevant information on the milking and the milking routine (Quinn *et al.,* 2004).

Therapeutic response of the cows can be monitored using individual somatic cell count data if available, or using the California Mastitis Test, and with bacteriological samples in herds with contagious mastitis. First choice antimicrobials for treating mastitis caused by streptococci and penicillin-susceptible staphylococci are β-lactam antimicrobials, particularly penicillin G. Broad-spectrum antimicrobials such as third or fourth generation cephalosporins should not be used as first alternatives for mastitis, as they may increase emergence of broad-spectrum β-lactam resistance. Systemic treatment is recommended in clinical mastitis due to *Staphylococcus aureus* and in severe cases of coliform mastitis, preferably in combination with intramammary treatment (Barkema *et al.*, 2006).

Too short duration of standard treatment is probably an important reason for poor cure rates in mastitis therapy. A longer treatment improves cure rates, and duration of treatment should generally be extended in mastitis caused by *Staphylococcus aureus* and *Streptococcus uberis* (Deluyker *et al*, 2005; Pyörälä, 2009).

Clinical mastitis should be treated for at least three days; this recommended treatment duration is longer than label treatments in many countries. All mastitis treatment should be evidence based i.e., the efficacy of each product and treatment length should be demonstrated by scientific studies (Cockcroft and Holmes, 2003).

Subclinical mastitis

Treating subclinical mastitis with antimicrobials is generally not economical during lactation because of high treatment costs and poor efficacy. In a study with a large number of subclinical mastitis cases, the overall bacteriological cure rate for antimicrobial treatment was

75% and that for no treatment 68% (Pyörälä, 2009). The marginal benefit applied for streptococcal mastitis only; in mastitis due to *Staphylococcus aureus*, antimicrobials were equal to no treatment. Treatment of subclinical mastitis will not affect the incidence of mastitis in the herd unless other preventive measures are taken. According to Hallén *et al*., (2008) and Pyörälä (2009) were reported that treating cows based on high somatic cell counts have generally shown that no effect on milk production. In herd problems caused by very contagious bacteria such as *Staphylococcus aureus* or *Streptococcus agalactiae* treatment of subclinical mastitis is advised (Wagner and Erskine, 2006).

Antimicrobial resistance

Public health consequences from the excessive use of antimicrobials in livestock production include the emergence of resistant microbes, which can then be transferred to humans through the food chain. Bacterial antimicrobial resistance has become a serious problem worldwide, and mechanisms of resistance have been identified and described for all known antimicrobials currently available for clinical use in human and veterinary medicine. Additionally, antimicrobial resistant bacterial pathogens in animals not only pose a risk with respect to animal health but are a growing concern with regard to possible transmission to humans as food-borne diseases (White and McDermott, 2001).

Different strategies for antimicrobial treatment will have different impacts on the development of antimicrobial resistance. Bovine mastitis is the single most common cause for antimicrobial use in lactating cattle worldwide (White and McDermott, 2001). There is a variety of antimicrobials that are used for the prevention and treatment of mastitis. Therefore, resistance to antimicrobials is expected. Bacterial cure rates for mastitis cases (e.g. *Staphylococcus aureus*) using antimicrobials are especially less than satisfactory, and seldom exceed 50% (Barkema *et al.,* 2006).

Antimicrobial resistance in gram-positive bacteria, typically found in bovine mastitis cases, is of concern due to less efficient antimicrobial options compared to gram-negative bacteria. According to Barkema (2008), resistance to penicillin is most common, where resistance to methicillin is most serious, because these strains are usually multi-resistant. Narrow spectrum

penicillins were introduced in the late 1940s and shortly there-after, resistance was observed. The rate of narrow-spectrum penicillin resistance varies per country and also over time within countries. For example, in the U.K., penicillin-resistance in *Staphylococcus aureus* isolated from bovine mastitis has increased from 2% in 1949 to approximately 70% in the 1980s (Aarestrup and Jensen, 1998).

2.6. Control and Prevention of Mastitis

2.6.1. Control of mastitis

Control of bovine mastitis is essential to produce high quality milk efficiently and to maximize profits. Udder hygiene practices are necessary components of a complete program for mastitis control (Pankey *et al.,* 1984). Therefore, post milking teat sanitation with an effective formulation significantly reduces rate of new intramammary infection by contagious pathogens *Staphylococcus aureus* and *Streptococcus agalactiae* and of minor mastitis pathogens such as Coagulase-negative staphylococci (CNS) (Pankey *et al.,* 1984). Post milking teat dips are designed to kill bacteria that contaminate teats during the milking process and to maintain or improve teat skin and teat end quality.

2.6.2. Monitoring of udder health status

Monitoring udder health status is an important principle of mastitis control. A regular quantitative assessment of udder health status is available through the use of SCC data (Radostits *et al.,* 1994). Establishing meaningful goals, and monitoring the progress towards attaining them over time, is a cornerstone of an effective udder health management program. Over the last decade, there have been tremendous developments in the tools and processes for monitoring udder health and milk quality in dairy herds. There has been a considerable development in the use of SCC data for monitoring udder health and milk quality (Schukken, 2003).

2.6.3. Prevention of mastitis

Prevention is the key to reducing the mastitis in the herd. If only the infected cows are treated, and no steps towards prevention are taken, there will be little or no reduction in the amount of mastitis in the herd (KCBS, 2005).

2.6.3.1. Improvement of milking practice

Milking procedures known to reduce the spread of contagious pathogens include the use of gloves by milkers, pre-dipping and post-dipping with a proven germicidal teat dip, drying teats with single-service paper towels or cloths, and disinfection of milking units after each cow with a back-flush system (John, 2003).

Udder washing

Adequate washing is important to prevent environmental mastitis, caused by coliforms and other microbes from contaminated environments. the lowest bacterial count in milk is obtained by washing the udder by using individual moist paper towels, wash and dry the teats only**,** wetting the udder and the teats results in more bacteria getting into the milk than if only the teats are wet (Pankey, 1989). Bacteria transmit to the uninfected from the contaminated hands of the milkers. Thus the milker's hands should be washed thoroughly with disinfected soaps before milking and clinically infected cows should be milked last. Teats should be cleaned and dried before milking (Mustefa, 2003).

Use of back-flush

Back-flushers have been developed to sanitize the liners and claws between milkings. Most units on the market have four or five cycles. The first cycle is a water rinse, followed by iodine or similar sanitizer rinse, a clear water rinse, and positive air dry cycle. Research has demonstrated that back-flushers do reduce the number of bacteria on the liners between cows, but do not reduce the number of bacteria on teats. Back-flushers also may stop the spread of contagious organisms, but this can also be accomplished at a much lower cost by teat dipping. There is no effect on environmental pathogens that are encountered between milkings (Schroeder, 2010).

Milking machine

The entering of micro-organisms, such as bacteria into the teat may be forced by milking machine, especially at the end of milking. Injured teats where the opening is to wide can be easily accessed by the bacteria (Christa, 2008).

One of the essential steps is the correct use of milking equipment and its periodic evaluation. This includes washing and disinfection of the milking machine and the production line before and after milking (Bogni *et al.,* 2011). Milking units that incorporate back-flush systems are designed to remove pathogens from milking units immediately after each cow is milked. Back-flush systems are used to prevent contagious pathogens from spreading from cow to cow via milking equipment (John, 2003).

2.6.3.2. Preventing mastitis with teat sanitizers

Effective and economical mastitis control programs rely on prevention rather than treatment. Herds practicing mastitis prevention produce higher quality milk at less cost than herds that do not (Ronald *et al.*, 2003). Sanitation has been advocated for the control and prevention of udder infections ever since the infectious nature of mastitis is confirmed. Over the years, sanitary measures which were not backed up by scientifically sound evidence have been recommended. These have failed unfortunately in preventing udder infections and have brought the concept of the usefulness of sanitation into disrepute (Newbould, 1965). Preservation of healthy teat skin is important in maintaining a natural defence against infection. Improvement or maintenance of teat condition is important to the dairy producer because it can affect the bacterial colonization of skin, milk let-down, milk-out time, milking speed and parlor through put (Thomas, 2002).

As stated by Pankey *et al.,* (1984), major emphasis was given to control udder infections by *Staphylococcus aureus* and *Streptococcus agalactiae.* Infected udders were recognized as the main reservoir of both *Staphylococcus aureus* and *Streptococcus agalactiae.* These pathogens were transmitted by hands and milking equipment. Number of new intramammary infections caused by *Staphylococcus aureus* and *Streptococcus agalactiae* was reduced significantly by disinfection of teat skin, milking equipment, and milkers hands. It was reported that incidence of intramammary infections was correlated to the number of mastitis pathogens on the teat end (Pankey *et al.,* 1984).

Results from numerous experimental and natural exposure studies indicated that many teat dip formulations reduced infection rate compared with no post-milking teat sanitation. *Staphylococcus aureus* and *Streptococcus agalactiae* are considered to be contagious forms of mastitis and are controlled most effectively by post-milking teat sanitation (Pankey *et al.*, 1984). Control of mastitis caused by environmental pathogens, esculin-positive streptococci and coliforms was not as evident by post milking teat sanitation (Smith *et al*., 1985).

Factors currently considered important in the control of mastitis caused by environmental pathogens include management and increased resistance of cows (Smith *et al*., 1985). One recommended approach to control was decreased exposure of teat ends to environmental pathogens. Pre-milking udder preparation methods were evaluated by Galton *et al*., (1984). Lowest bacterial counts were obtained when teats were cleaned with a water hose (pipe) or wet towel or when a pre-milking disinfectant teat dip followed by drying with paper towels was used. Thorough drying of teats with paper towels after dipping with an iodophor sanitizer was necessary to reduce iodine residues in milk. In addition, use of 0.5% iodophor sanitizer caused less iodine residue in milk than a 1% iodophor (Galton *et al*., 1984).

Teat dipping

Dips can be applied by hand-held cups or with a 'power dipper' (a dip cup on a wand with solution applied when a trigger is activated). Cups should be emptied before refilling, rather than 'topped up' when the solution becomes low and any solution remaining at the end of milking should be discarded. The cup should be large enough to accommodate the teat without causing excessive spillage of the disinfectant solution. The act of immersing each teat in a reservoir of disinfectant usually ensures the entire teat (any area in contact with the teat liner) will be covered, as long as the cup is deep enough and filled with the appropriate amount of an effective solution (Richard, 2010). Teat disinfection is one of the most important preventive measures in mastitis control. It is carried out either immediately prior to milking (pre-dipping) and/or, most commonly, after milking (post dipping) (Roger and Peter, 1996).

I) Pre-milking dipping

The primary objective of pre-milking udder preparation and teat sanitization is to achieve an acceptable level of decontamination of teat skin. This aids in reducing the spread of micro organisms and incidence of new intramammary infection, and in minimizing the number of bacteria that find their way into the raw milk supply (Nickerson, 2001). Pre-milking teat disinfection involves applying a quick-acting disinfectant just before milking to reduce the population of mastitis-causing bacteria on teat skin especially in the region of the external teat orifice. The major effect of pre-milking teat disinfection is therefore against those environmental micro-organisms which cause mastitis. Pre-dipping reduces new environmental streptococcal infections and *Escherichia coli* by as much as 50%. Pre-milking dipping teats should be considered if there are high numbers of mastitis cases due to environmental bacteria (> 5 per 100 cows per month), or, particularly in spring-calving cows, at high risk periods such as in the first week after calving. Pre-milking dipping needs a minimum contact time of 30 seconds and must be wiped off the teats with single use towels(one per cows) prior to the application of the milking unit (Richard, 2010).

II) Post milking dipping

The role of post milking teat dipping as a management tool in preventing new intramammary Infections in dairy cows are well documented (Pankey *et al.*, 1984). Post milking teat dipping is the most effective milking hygiene practice for preventing new infections caused by the two most common contagious mastitis organisms, *Staphylococcus aureus* and *Streptococcus agalactiae* (Nickerson, 2001). Post-milking teat disinfection should prevent mastitis and enhance teat skin condition. KENOSTART™ is demonstrated to be efficient against bacteria causing mastitis. It is tested according to European Standards EN 1656 (field conditions) against *Staphylococcus aureus, Escherichia coli, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis* and *Corynebacterium bovis*, (CID LINES, 2007).

In preventing mastitis, the post-milking teat disinfection works by the removal of mastitis-causing bacteria from the teat skin and teat sores. Disinfectant should be applied as soon as the cluster is removed, while the teat canal is still open. The dip can then penetrate the teat orifice, ensuring that those bacteria which have just entered the canal will also be killed. Post-

milking teat disinfection, dipping or spraying, removes the bacteria that spread during milking and, as such, is an extremely effective weapon against the spread of contagious mastitis (Richard, 2010).

Teat disinfection products

More than 10 different active ingredients have been used in teat disinfectants throughout the world over the past 20 years. The National Mastitis Council in the United States has reviewed and summarized all the scientific literature on teat disinfectants published since 1980 (National Mastitis Council, 2001). The most commonly used active ingredients are: iodine, chlorhexidine, quaternary ammonium compounds, hydrolyzed fatty acids, hypochlorite, and acid anionic compounds. Hence, emollients are added to the disinfectant preparations. Teat skin has relatively few sebaceous glands and continual washing followed by exposure of damp teats to a cold and windy environment can remove protective fatty acids and lead to cracking. Thus, the addition of emollients, such as glycerine, sorbitol, lanolin or propylene glycol, to teat disinfectant can improve teat skin health and so reduce the likely reservoir of mastitis bacteria in teat sores and cracks. Many teat disinfectants contain emollients when they are sold. Addition of excessive amounts of any emollient (i.e. >20%) will most probably reduce killing activity and could lead to increases in new mastitis cases (Richard, 2010).

Some of teat disinfectants used in teat dips

Antimicrobials used in teats include iodine (most common), chlorhexidine, surfactants (such as linear dodecyl benzene sulfonic acid), organic acids, quaternary ammonium salts, and acidified sodium chlorite (ASC).

- Iodophor

Iodine is a broad spectrum germicide, which is fast acting and effective against all mastitis causing bacteria as well as fungi, viruses, and bacterial spores. This element is microbicidal due to the oxidizing reaction between iodine and organic matter. Iodine is dissolved in water by complexing with water-soluble detergents or surfactants, and this resulting solution is referred to as an iodophor (Nickerson, 2001).

Iodophors formulations kill bacteria by non-biological chemical action, through a redox mechanism with quick action. They are considered relatively non-toxic but should be used according to the manufacturer's recommendations as they may develop irritation (Barkema, 2008). Recognized as an antiseptic and disinfectant effective by having a broad spectrum of antimicrobial activity against vegetative bacteria, fungi, viruses and spore-forming bacteria (King, 1981), but has several properties that make it difficult to use as a low solubility in water, irritant in alcoholic solution and its strong odor (Barkema, 2008), these problems are treated with the combination of reducing iodine solubilizing agents.

- Quaternary ammonium compounds

Teat germicides containing quaternary ammonium compounds are Alkyl dimethyl benzyl ammonium chloride and alkyl dimethyl benzyl ammonium bromide which used as germicides, these formulations are usually composed emollients, water-soluble colouring agents, thickening agents and carriers. Quaternary ammonium compounds are microbicidal through denaturing cell proteins, inhibiting enzyme systems, and altering membrane permeability, leading to bacterial cell disruption (Nickerson, 2001). These products are safe and relatively non-toxic. Although Fustes *et al.,* (1985) found that benzalkonium chloride solution at 1 and 1.5% were serious disturbances in the teat skin characterized by erythema, dehydration intense epithelial sheet loss and pain are not corrosive to equipment and rapidly degrade in the environment. It is imperative that dip cups be cleaned periodically during milking if they become overloaded with organic material as Serratia species and Pseudomonas species have been known to survive in quaternary ammonium teat dips (National Mastitis Council, 2001).

- Acidified sodium chlorite (ASC)

The ASC system derives from the combination of sodium chlorite and a suitable acid such as lactic acid or mandelic acid, forming the active microbicidal components, chlorous acid and chlorine dioxide. Both of these compounds have broad spectrum of action and are effective against Gram-positive and Gram-negative bacteria, as well as moulds, yeasts, and viruses. Acidified sodium chlorite products include humectants and emollients as ingredients, and are generally two part systems composed of an activator and a base, which must be mixed and

prepared daily to provide optimal antimicrobial activity. The mixed product contains a sodium chlorite level of approximately 0.32%. After application, the product dries on the surface of the teat skin, forming a barrier, in which the killing action of chlorous acid is maintained (National Mastitis Council, 2001).

- Dodecyl benzene sulfonic acid (DDBSA)

Teat dip products containing DDBSA incorporate an anionic surfactant as the active ingredient along with an organic acid, glycerin, and other emollients. It is believed that DDBSA products function by denaturing the proteins of microbial cells, inactivating essential enzyme systems, and disrupting cell membranes. Teat sanitizers composed of DDBSA are effective against Gram-positive and Gram-negative bacteria as well as yeasts, and are available as conventional or barrier formulations (Nickerson, 2001).

- Chlorhexidine

Chlorhexidine is a rapidly acting, nonirritating germicide composed of biguanide compounds. This germicide is effective against most Gram-positive and Gram-negative bacteria as well as some viruses by precipitating cytoplasmic proteins and macromolecules. However, if heavily contaminated, Serratia species and Pseudomonas species can survive in chlorhexidine-based products and serve as potential mastitis pathogens. Teat sanitizers utilizing this germicide contain between 0.35 to 0.55% chlorhexidine gluconate or acetate as well as humectants and emollients to minimize irritation. Chlorhexidine sanitizers adhere well to teat skin, provide antimicrobial activity over time, and do not have deleterious effects on teat skin. Both conventional and barrier formulations are available (National Mastitis Council, 2001).

- Other disinfectants tested

Other studies have developed chlorous acid and chlorine dioxide showed a reduction in new infections by Streptococcus dysgalactiae and other pathogens causing mastitis (Oliver *et al.*, 1989; Harmon, 1996). Furthermore, Armenteros *et al*., (1998) reported the effectiveness of UDERTAN (post-milking disinfectant breast natural origin) and their advantages in terms of safety for the teat skin accelerates wound healing and has no problem of residues in milk.

Major limitations

Teat disinfection may prevent new infections, but does not reduce the duration of existing infections some infections persist for months or years, and when applied as a measure only disinfection is required several months to reduce the level of infection (Harmon, 1996). A study showed that a 50% reduction of new intramammary infections reduced by 14% of infected quarters in 12 months (Dodd *et al.*, 1969).

2.7. Status of Bovine Mastitis in Ethiopia

In Ethiopia, the disease is inadequately investigated, and information related to its magnitude, distribution, and risk factors is limited. Such information is important to predict when designing appropriate strategies that would help to reduce its prevalence and effects. In different parts of the country, the information on Table 3 points out that bovine mastitis is one of the most frequently encountered diseases of dairy cows.

Table 3: Prevalence of bovine mastitis in different parts of Ethiopia

Study area	Name of the Author and year of study	Overall prevalence of bovine mastitis in %
Arsi	Takele (1987)	53.00
Debre-Zeit	Geressu (1989)	39.50
Soddo	Shimelis (1990)	45.90
Dire-Dawa	Darsema (1991)	36.90
Chaffa valley	Fekadu (1995)	38.60
Addis Ababa	Lemma *et al*., (2001)	30.80
Welayta and Sidama Zones	Biffa *et al*., (2005)	34.90
Sebeta	Sori *et al*., (2005)	52.78
Asella,	Lakew *et al*., (2009)	64.60
BahirDar and its Environs	Molalegne *et al*., (2010)	28.20
Holeta	Mekibib *et al.*,(2010)	71.00

Generally these limited studies showed that bovine mastitis is among the problems that hinder dairy productivity in Ethiopia and this requires the development of methodologies of control program under the prevailing husbandry system. However, according to Hussein *et al.* (1997), so far efforts have been concentrated only on the treatment of clinical cases. On the other hand, losses from mastitis have been attributed mainly to decreased milk production from subclinical mastitis (Degraves and Fetrow, 1993).

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was conducted in Holeta research centre dairy farm, Holeta town, from June to December 2011. Holeta town is located at 40 km west of Addis Ababa at elevation of 2400 meters above sea level in the central Ethiopia. The area is characterized by mild subtropical weather, with an average minimum and maximum annual temperatures of 6.3^0C and 22.1^0C, respectively. The area has also experienced bimodal rain fall pattern with a long rainy season extending from July to September while the short rainy season extends from March to April (CSA, 2004).

3.2. Study Design

An experimental Randomized Controlled Trials were used to assess the effect of KENOSTART™ and KENO™PURE teat disinfectants on lactating cross breed dairy cows in the Holeta research center dairy farm, central Ethiopia.

3.3. Study Population

All lactating cows which were kept under controlled management, i.e., cows which received the same husbandry systems and negative for CMT were considered as the study animals. Animals which mismatch to the rest of the group i.e. the study didn't include calves, male animals, non-lactating cows, local breeds and others. Cows which have open lesions and abnormalities on the udder were also excluded from the study.

3.4. Sample Size Determination

The sample size for binary outcomes were determined by using the following formula which is described by Kenneth and David (2005), by assuming α=0·05, power=0·80 (table value = 7.85), and equal sample sizes were used in the two groups (i.e. treated vs. untreated).

$$n = \frac{\text{Power } [(R+1)-p2\,(R^2+1)]}{p2\,(1-R)^2}$$

$$n = \frac{7.85[(1.5)-1.01125)]}{0.809\,(0.5)^2}$$

$$n = \frac{7.85*0.48875}{0.202} = \frac{3.837}{0.202} \qquad R = \frac{P1}{P2} = \frac{0.4045}{0.809}$$

n = 19 R= 0.5

Where;

n = the sample size in each of the groups

p1 = event rate in the treatment group (not in the formula but were applied when R and p2 are estimated i.e it was expected to reduce to 40.45% which is half of the previous prevalence)

p2 = event rate in the control group in the study area; the overall prevalence of mastitis in the farm is 80.9% which has been taken from previous unpublished paper.

R = risk ratio (p1/p2) were determined.

Thus, the total sample size was 38 lactating dairy cows, and in each group 19 lactating dairy cows were randomly allocated.

3.5. Study Animal Selection

A total of 38 lactating cross-breed dairy cows were randomly selected from those which fulfilled the inclusion criteria (n = 51) and then were randomly allocated in to treatment and control group. Then, cows involved in these groups were coded.

3.6. Study Variables

The dependent variables were incidence of mastitis, teat-skin and teat-end condition and cost for clinical mastitis treatment between study groups. The independent variable was treatments (treated and control groups).

3.7. Study Methods

3.7.1. Screening the study animals

The Holeta research center dairy farm had 105 lactating dairy cows at the time of the study; among these, 40 animals were excluded from the study because of having open lesions on their teat and those which were nearly to become dry off. The remaining 65 dairy cows were screened by CMT and out of these, 31 were negative (In this study, CMT were used as a parameter to detect subclinical mastitis, threshold= score 0: no mastitis; score traces 1, 2, 3: mastitis) and 34 were positive for CMT test. Those positive cows were segregated and treated with Lactaclox infusion; combined preparation of ampicillin and cloxacillin (Norbrook Laboratories Ltd, Station Works, Newry, Co.Down, N. Ireland, BT35 6J) against clinical mastitis for five days; and then, after 20 days they were again checked by CMT and 20 cows were become negative for CMT test and the rest 14 were found positive which were excluded from the study. At the end, a total of 51 (31+20) dairy cows were fulfilled the inclusion criteria of being negative for CMT test.

3.7.2. Teat end and skin condition scoring

Teat end and teat skin condition of each study cows were scored every 20 days (Josephine V., personal communication) based on visual and tactile observations following characterization of Neijenhuis *et al.,* (2004). The results were scored:

For teat skin condition scores; teat skin is smooth, free from scales, cracks, or chapping=1; teat skin shows some evidence of scaling=2; chapped with some small warts=3; chapped and cracked. Redness, indicates the presence of inflammation=4.

For teat end condition scores; teat end sphincter is smooth, with no evidence of irritation=1; raised ring=2; roughened with slight cracks, but no redness is present=3; the sphincter inverted with many cracks, giving a ‘flowered ‘appearance=4; and severely damaged with ulceration, scabs or open lesions=5. The status of the quarters, teat skin and teat end condition were determined and recorded before the study begun (Fig 1 & 2).

The number of eligible quarters examined during the trial was presented in Table 4.

Table 4: Number of quarters examined in each group, June 2011.

Study groups	Number of quarter in each trial	Total number of quarter in all trial (1st to 7th trial)	Total blind teat	Total number of quarters examined in both groups
treated	75	525	1	1043
control	74	518	2	

3.7.3 Product description and teat dip application

KENOSTART™ (brand name) (CID LINES, Lot 014073, Belgium) was an experimental dip which consists of stable iodine as an active pharmaceutical ingredient; ready to use and applied as post dip. Characterized by its concentration: 3000 ppm, pH: 4.0 – 6.0, Density (20°C): ca. 1.020 kg/l, Viscous dark brown solution in its appearance and, complete solubility in water. It has film forming around the teat; due to its non-dropping (uniform barrier), healing and skin conditioning properties. It is non acidic and contains high amount of emollients for maximum teat conditioning. The instruction of the manufacturer was followed accordingly: The dip cup which holds 300ml of dip was used for application of teat dip after milking until it covers three quarters of teat length. The dip cup replenished as necessary and emptied after treatment and washed before reuse. The product is used as a post-milking teat dip two times per day and can be used in lactating and pregnant cows. It has zero withdrawal periods for milk, meat & offal.

KENO™PURE (brand name) (CID LINES, Lot 014401, Belgium) was used for the pre-milking dip and it consists of lactic acid as an active pharmaceutical ingredient. The advised method of application for the research trial is with a foaming dip cup. The solution required for the foam dip cup application was 40%, i.e. mixing 400ml of KENO™PURE with 600ml water. In concentrate form, the product has pH value: ca. 2.25, density (20°C): ca. 1.06 kg/l and it has liquid physical state, green in its appearance and completely soluble in water.

Two milkers were selected and trained on the application of teat dipping drugs. Before milking, all quarters of the teats of cows in study groups were cleaned with warm water by sponge and then dried with individual towels. The dipping cup was filled with 40% KENO™PURE solution then all quarters of the treated group were dipped full length with this pre-milking solution (foam) and waited for 30 seconds then wiped with individual towel so that complete milking were allowed. After milking, the ready-made KENOSTART™ teat dip was poured in to its own dipping cup then applied in all quarters of treated cows and left to dry. This teat dipping procedure was performed twice a day with respect to the milking time (3PM in the afternoon and 3AM after midnight). Like teat skin & teat end conditions was scored, the incidence of mastitis at quarter level were checked by CMT in every 20 days.

3.7.4. Management of the study animals

The cows were housed and managed under normal procedures used by the research center. Cows were housed in free stalls bedded with sawdust or dairy waste solids. The farm had semi-intensive management system, the cows were often provided with some supplementary diet in addition to the natural pasture and agricultural by-products. The Farm had ten milking machines which were used for milking; however, no disinfectants have been utilized or back-flushed between different rounds of milking time.

3.8. Financial Loss Due to Treatment of Clinical Mastitis

The direct financial loss was thus computed according to the formula adopted from Singh and Singh (1994), as follows:

Treatment loss = Average cost of treatment per day X Animal mastitis days

$$\text{Average cost of treatment per day} = \frac{\text{Total price of medicines used for treating mastitis}}{\text{Animal mastitis days}}$$

3.9. Data Quality Control and Assurance

The following measures were taken to ensure the quality of the study:

- Standard / literature based procedures were followed.
- Control animals were taken so that variations between groups were investigated.
- Quality and analytical grade CMT reagent was used.

3.10. Data Management and Analysis

All the collected data were entered to Microsoft excel 2007 spreadsheet then transferred to SPSS software version 16 for analysis. Descriptive statistics and incidence rate were computed to present the results. Chi-square test was used to compare the effect of pre and post teat disinfectants in terms of incidence of mastitis and improvement of teat skin and teat end condition between treated and untreated animals. All analysis was performed at 5% significance level.

4. RESULTS AND DISCUSSION

RESULTS

4.1. Efficacy of KENO™PURE and KENOSTART™ teat disinfectants on reducing the incidence of mastitis

A greater number of new intramammary infections were found in the control group than in the treatment group. One hundred fourteen (22%) quarters out of 518 in the control group and 42 (8%) quarters out of 525 in the treatment group were positive for new intramammary infection. The incidence rate at cow level shows a significant variation ($P < 0.05$, $\chi^2 = 41.592$) between treated 23 (17.3%) and control 65 (49.6%) groups respectively. The incidence of clinical mastitis occurred at quarter level in dipped cows was 2 (0.4%) as compared to non-dipped ones which was 7 (1.4%), ($P > 0.05$ $\chi^2 = 3.039$).

Table 5: Comparison of the incidence of mastitis at cow level between dipped and non-dipped animals in each trial, June to December 2011.

Trial number	Incidence in control group (%)	Incidence in treatment group (%)	Chi-square values (χ^2)	P-values
Trial-1	36.8	52.6	0.958	0.328
Trial-2	42.1	15.8	3.199	0.074
Trial-3	42.1	10.5	4.886	0.027
Trial-4	47.3	5.2	8.686	0.003
Trial-5	52.6	10.5	7.795	0.005
Trial-6	68.4	15.7	10.795	0.001
Trial-7	59	10.5	9.471	0.002

4.2. Effectiveness of KENO™PURE and KENOSTART™ teat disinfectants on improving the teat skin and teat end condition

In this trial, KENO™PURE and KENOSTART™ teat disinfectants provided better teat skin and end condition to the treatment group compared to the non-dipped controls (Fig. 1 & 2). Cows in the treatment group had shown a greater number of smooth teat skin conditions ($P<0.05$, $\chi^2 = 257.0$) than cows in the control group and they were also revealed significant result in the reduction of scaly ($P<0.05$, $\chi^2 = 168.0$) and chapped teats ($P<0.05$, $\chi^2 = 19.759$). In addition the teat skin inflammation was decreased ($P<0.05$, $\chi^2 = 10.795$) (Fig.1).

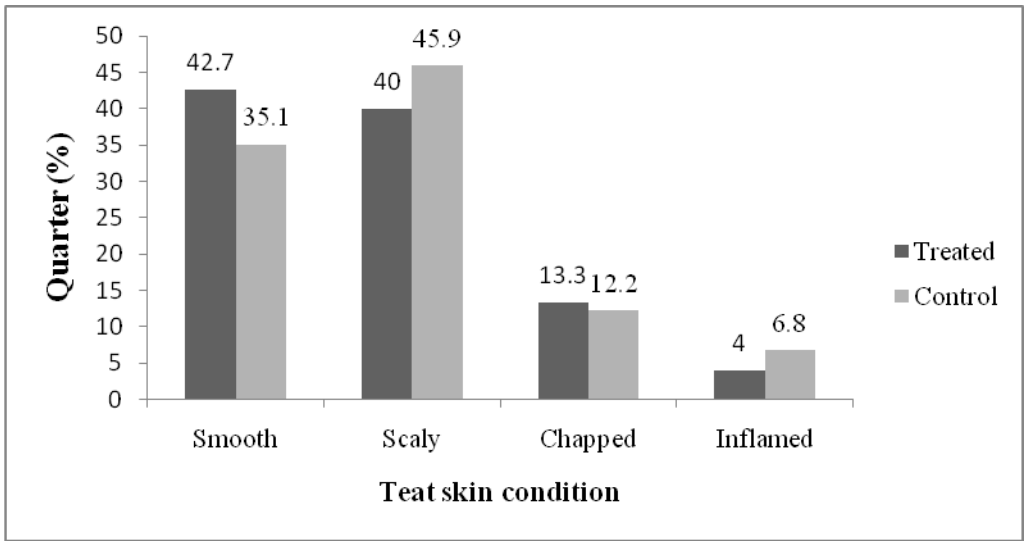

Fig. 1: Teat skin condition of study animals before the study begun, June 2011

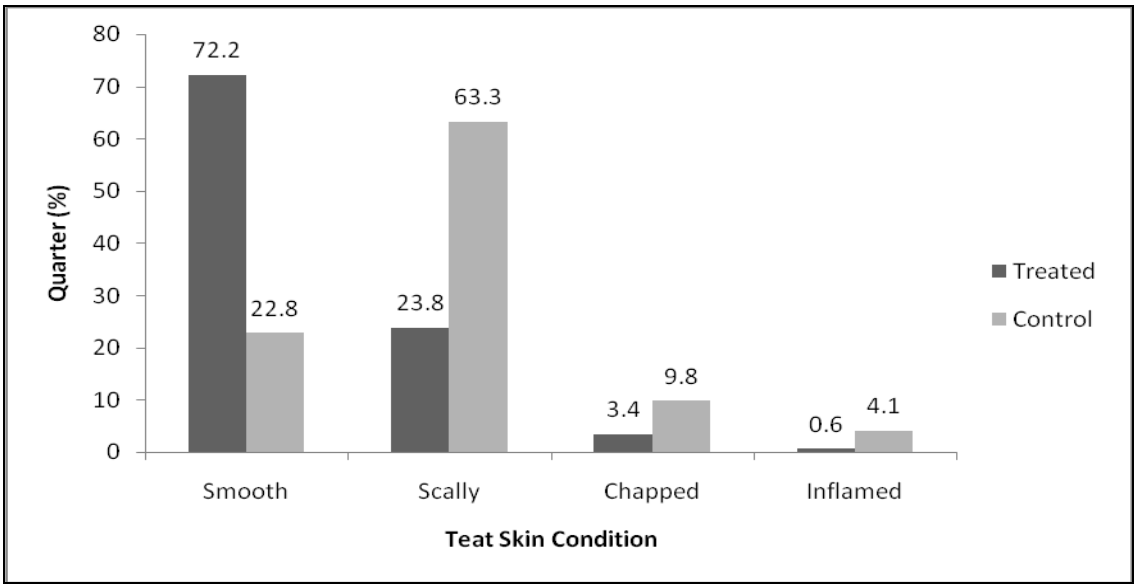

Fig. 2: Teat skin condition of study animals after treatment, June to December 2011.

There was also a statistically significant difference ($P < 0.05$, $\chi^2 = 189.3$) in teat end conditions between the study groups; the treated group had more smooth sphincter teat ends than the control group. In treated cows, the raised ring teat was greatly recovered ($P < 0.05$, $\chi 2 = 170.2$), the roughness of teat end were got better ($P < 0.05$, $\chi^2 = 4.439$) and inflammation of teat ends were eliminated ($P<0.05$, $\chi^2 = 6.366$) (Fig. 2).

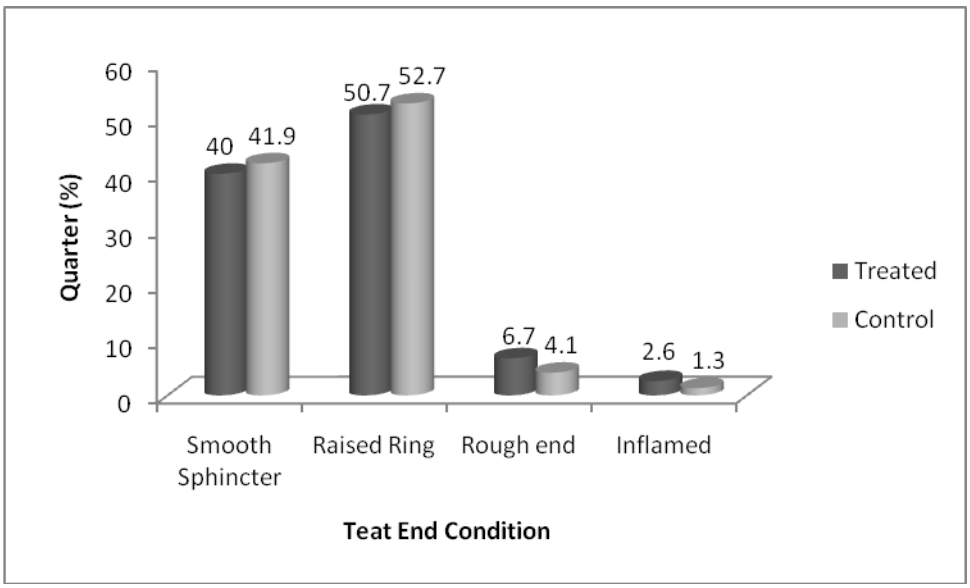

Fig. 3: Teat end condition of study animals before the study begun, June 2011.

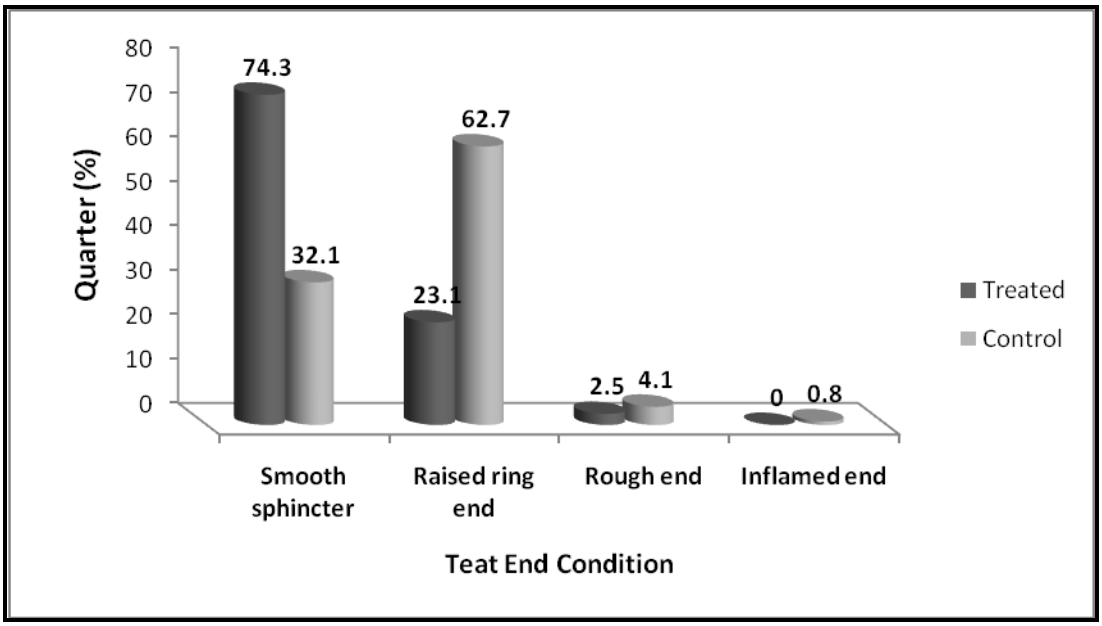

Fig. 4: Teat end condition of study animals after treatment, June to December 2011.

4.3. Financial Loss Due to Treatment of Clinical Mastitis

The direct financial loss due to clinical mastitis in treatment and control group was calculated to be 700 and 2450 ETB respectively (Table 6).

Table 6: Financial loss due to treatment of clinical mastitis in treated and control groups, June to December 2011.

Study groups	Noof infected quarters	Animal mastitis(days)	Unit price of the drug	Total price of drugs	Treatment loss (ETB)
treated	2	10	35	700	700
Control	7	35	35	2450	2450

DISCUSSION

This study was carried out to evaluate the efficacy of KENO™PURE & KENOSTART™ teat disinfectants on reducing the incidence of mastitis between the study groups. The overall incidence of mastitis at quarter level between treatment and control groups showed a significant difference ($P<0.05$, $\chi 2 = 41.592$). Likewise, the incidence rate at cow level also illustrated to have a significant variation between treated and control groups ($P <0.05$, $\chi^2 = 31.222$). This result shows that the combined (pre and post dipping teat disinfectants) effect of disinfecting drugs in dipped cows had more preventive effect than non–dipped cows. Previous studies showed that dipping teats of dairy cows before and after milking with an appropriate germicidal preparation was used to reduce teat skin colonization and contamination with mastitis-causing bacteria and minimize penetration into the teat canal (Nickerson, 2001). In support of this finding, Oliver *et al.* (1989) reported that the efficacy of chlorous acid and chlorine dioxide teat dip for the prevention of new intramammary infection reduced the infection of major mastitis pathogens by 24% in quarters of dipped ones, as compared to quarters in control group (76%). On the other study, significant difference in the efficacy of the experimental teat dip against *Staphylococcus aureus* were recorded comparable with the results observed in natural exposure studies that evaluated iodophor (Eberhart *et al.*, 1983; Nickerson *et al.*, 1986). This study is also in agreement with Kingwill *et al.* (1979) which demonstrated the benefits of post milking teat disinfection in reducing mastitis. On the other hand, Fox (1991) and Fox and Norell, (1994) reported that, the concentration of *Staphylococcus aureus* recovered from teat skin that swabbed with the disinfectant solution was lower in dipped teats as compared to untreated one. In this finding, the incidence of mastitis by using KENOSTART™ were reduced and this is in agreement with Nickerson, (1989) who reported that, the incidences of *Staphylococcus aureus* and *Staphylococcus epidermidis* intramammary infection were higher in herds using linear dodecyl benzene sulfonic acid (LDBSA) than in those using iodine.

The study was also assessed the incidence of mastitis in each trial, however, the incidence of mastitis at cow and quarter level in the first and second trials, were not significant ($P >0.05$, $\chi^2 = 1.6889$ and $P> 0.05$, $\chi^2 =2.1714$) respectively. This might be because of incorporating

negative cows in the study after they have screened for mastitis; thus the incidence between groups didn't have much variation at early stage. Obviously, teat dipping is a simple, effective, and economical means to reduce bacterial populations on teat skin both before and after milking, and an abundance of published evidence shows that this practice may reduce the rate of infection among dairy cows (Nickerson, 2001). However, if any one considers comparing the incidence rate from the time of introduction of dipping, it may take few months before the herd level of infection is reduced after teat dipping is initiated (Table 5).

The incidence of clinical mastitis occurred at quarter level in control group is higher (1.4%) than in treated ones (0.4%), nevertheless, it was not statistically significant ($P>0.05$). Although published data were not available regarding KENO™PURE and KENOSTART™, this finding is in agreement with Gleeson *et al.,* (2006), who reported the benefit of chlorohexadine solution which reduces the clinical cases, accordingly the number of cases of clinical mastitis at quarter level was higher for non disinfected teats (22%) compared to disinfected teats (6%). The combined effect of KENO™PURE and KENOSTART™ teat dip on preventing clinical mastitis was more in treated than control group. This reduction in incidence of mastitis was observed without disinfecting the milking machine; if the milking machine were disinfected the teat dip would have been more effective to control both subclinical and clinical mastitis at herd level.

The effectiveness of KENO™PURE and KENOSTART™ teat disinfectants on improving the teat skin and teat end condition were also assessed **(**Fig. 2 & 4**).** In treated groups, teat dips were increased the smoothness of the teat skin and reduced the scaly and chapped teats. This is due to the product KENOSTART™ has contained excipients such as Glycerol (draw water on to teat), Sorbitol 70% (softens the teat), Sodium Bisulphite 40%, Ethoxylated Lanolin 50% (coats the teat & retains moisture) (CID LINES, 2007). In addition, teat dips significantly reduced ($P<0.05$) the teat skin inflammation. This healing and skin conditioning were observed because of the disinfectant that forms a film around the teat due to its non-dropping properties (uniform barrier) (CID LINES, 2007).

Furthermore, in treated cows, the teat dip also recovered the raised ring and the roughness of teat end. Even though there was a difference in the recovery of roughness of teat end between the two groups, the variation was not statistically significant (P>0.05). This might be due to the slow recovery rate of the necrotized part of tissue. Besides, the teat dip improved the inflamed teat ends and significantly increased the smoothness of the sphincter (P<0.05). A smooth muscle sphincter surrounds the teat canal holding it closed, thus preventing the leakage of milk and serving as the body's first line of defence against intramammary infection (Neijenhuis *et al*., 2001). Usually after milking, this sphincter remains dilated for 1–2 hours, allowing bacteria to enter the teat canal (Nickerson *et al*., 1996; Neijenhuis *et al*., 2000). Therefore teat-ends with a shape that allows the sphincter to remain dilated for longer periods are likely to predispose cows to intramammary infection.

Chapped and rough teat skin is more susceptible to colonization by *Staphylococcus aureus* (Fox *et al*., 1991). If greater numbers of *Staphylococcus aureus* are able to persist on the teat skin, risk of the occurrence of new intramammary infection is higher (Neave *et al.,* 1969). Dry, cracked teat skin also collects more soil, which further reduces teat sanitation (Bushnell, 1985). When teats had the worst teat condition, milk yield was lowest and milk-out time was highest. As teat condition improved, milk yield increased and milk-out time decreased. Decreased milking time and increased milk yield provide additional economic incentive to maintain healthy teat condition (Thomas, 2002).Therefore, the management of teat antiseptics to maintain healthy teat skin is important for the welfare of the cow, both for cow comfort and to maximize the effectiveness of teat dips in the prevention of intramammary infection. According to Burmeister *et al.*, (1998), teat skin condition tended to improve over time. The teats dipped with UDDERGold had consistently improved ($P < 0.05$) skin condition by week 2 and through week 5 as compared with the skin condition during treatment week 1. The teat ends of teats treated with UDDERGold showed improvement ($P< 0.05$) by the 2nd week of application. Teats treated with QuarterMate (0.1% iodine) and 4XLAR (2.64% lactic acid and 0.64% sodium chlorite) showed to significantly ($P < 0.05$) improve teat end condition by the 3rd week of treatment application. These teats continued to improve and had the best teat end condition scores by the end of the experiment. In agreement with Burmeister *et al.*, (1998), the teat dips (KENO™PURE (Lactic acid<10%) and KENOSTART™ (0.3% iodine) of this

study were effective in improving the teat skin and teat end conditioning of the treated groups after treatment application. This trial was done by allocating the animals in to big two groups (treatment and control); however, it would have been more effective if the teat disinfectant was investigated within cow. Furthermore, lack of approximately similar age or parity of the studied dairy cows was another limitation of this study.

In this study, an average estimated financial loss due to treatment of clinical mastitis cases in treatment and control groups was 700 ETB (40.74 USD) and 2450 ETB (142.59 USD), respectively. This is a substantial loss in small holder dairy farms in Ethiopian condition. The estimated financial loss in control groups is more than (1075 ETB) an average adult income per year in rural areas to escape poverty (MoFED, 2010). The financial loss recorded due to treatment of clinical mastitis in this work was higher compared studies done in other countries. Accordingly, in Czech Republic in which the direct financial loss due to clinical mastitis was estimated to be €43.63 to 84.84 per cow per year within farm (Wolfova *et al.*, 2006). This experiment revealed the economic importance of the teat dip which has reduced the cost of treatment in the case of treated animals. It means that the use of these teat dips has reduced the cost of treatment of clinical mastitis by 71.43%. This reduction of financial loss with teat dipping will alleviate the problem of the farmers by increasing the dairy farm profitability.

5. CONCLUSIONS AND RECOMMENDATIONS

The use of pre- and post-milking teat antiseptic is probably the most important management strategy to reduce the new intramammary infection rate in dairy cows and to maintain a low level of mastitis. This practice, along with use of proper milking technique including disinfection of the milking machine will help to reduce new cases of intramammary infection.

- ✓ The incidence of subclinical mastitis was significantly lower in treated animals than in controls. In addition, the incidence of clinical mastitis was also considerably reduced in case of treated animals. So, from the present study it can be concluded that the teat dips could be highly effective in preventing mastitis.
- ✓ Teat dips were improved the teat skin and teat end condition of treated animals with maintaining the healthiness of the udder. The trials have shown the usefulness of teat dip (for instance healing chapped teats) on various condition of teat skin and teat end. Thus, it can be concluded that the drug would get the teat skin and teat end conditions to be better.
- ✓ The financial loss incurred due to the treatment of clinical mastitis cases in non-treated animals was significantly higher as compared to the treated ones; indicating the economic importance of the teat dip in reducing the cost of treatment in the case of treated animals.

Based on the above conclusions, the following recommendations are forwarded:

- ✓ Teat dips have to be introduced in to the dairy farms as part of the mastitis prevention and control programs. Moreover, the application of these disinfectants is recommended as they were significantly reducing the financial loss due to treatment of clinical mastitis.
- ✓ As the reduction in incidence of mastitis was without disinfecting the milking machine, it is recommended to study the effect of disinfecting the milking machine along with teat dips. It is expected that, if the machine is disinfected, the teat dip would have been more effective.
- ✓ Besides, these disinfectants have to be investigated under hand-milking condition and within cow by using split udder design.
- ✓ The effectiveness of other teat disinfectants has to be investigated in the future as a part of prevention and control of bovine mastitis.

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7. APPENDICES

Appendix I

CMT scoring chart

Trial number: _____ Date: _____

Cow Id	Cow Code	Left Rear	Left Front	Right Rear	Right Front	Remarks

Appendix II

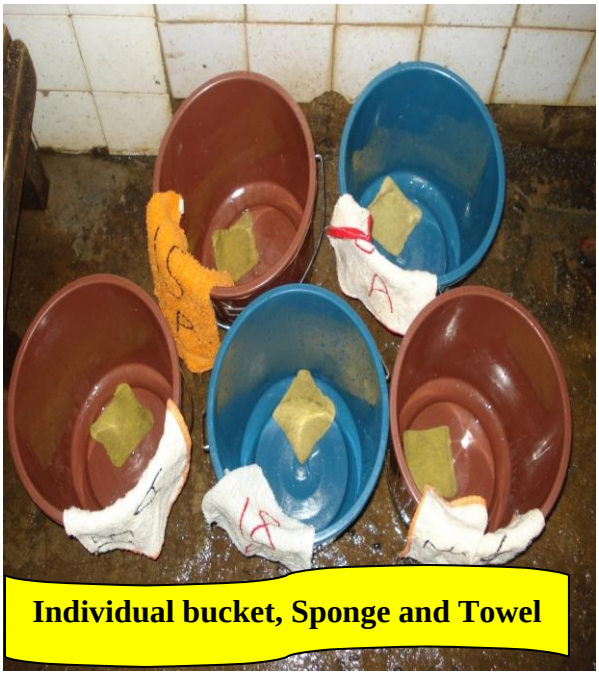
Teat condition scoring chart

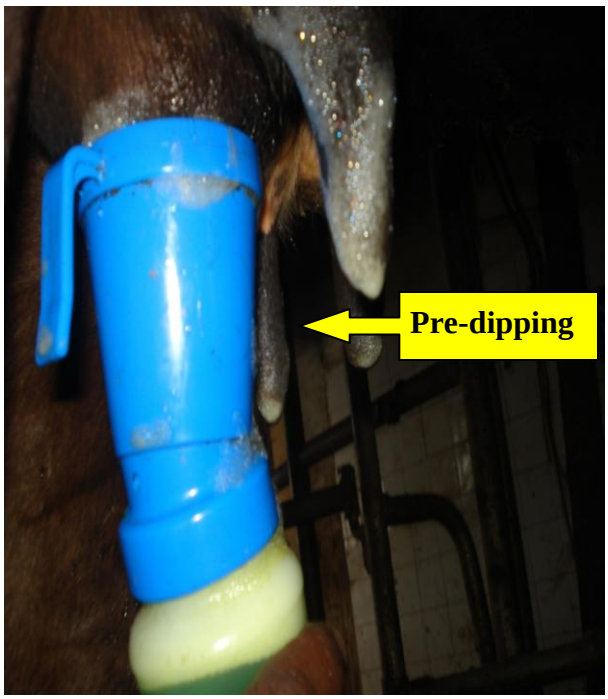
Trial number: _____ Date: _____

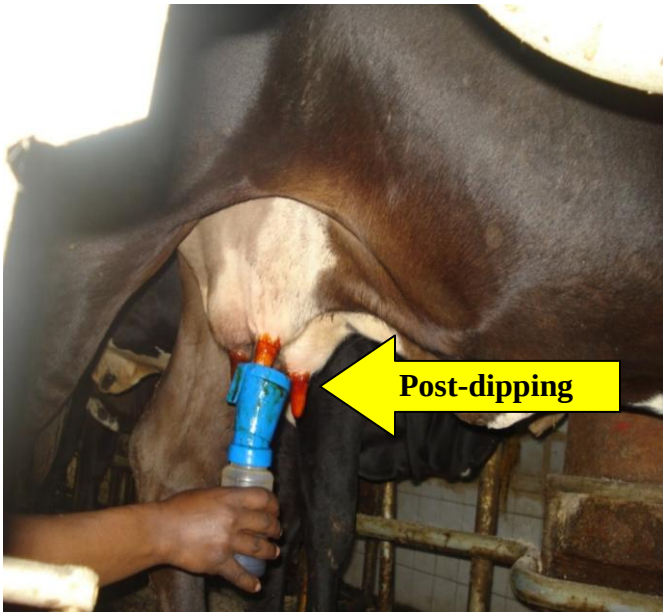
Cow Id	Cow Code	Left Front		Right Front		Left Rear		Right Rear	
		Skin	End	Skin	End	Skin	End	Skin	End

Appendix III

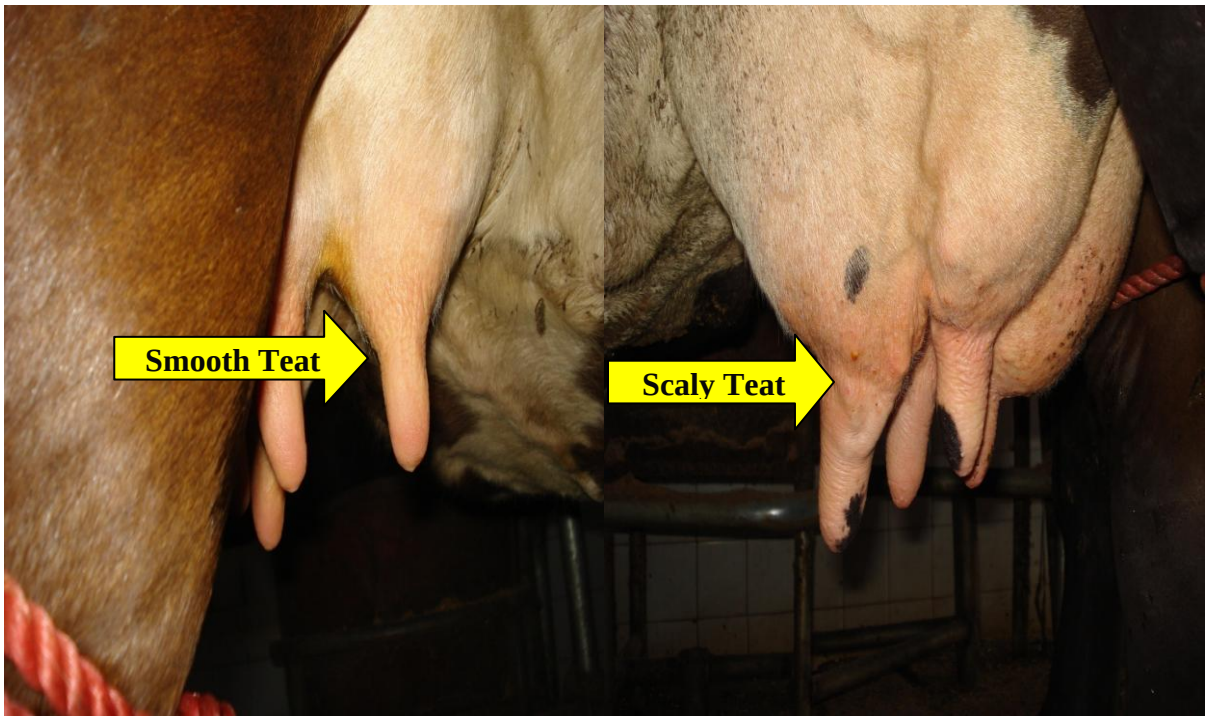
Sample Pictures







Teat conditions of the animals



Reagents

The reagents used for the experiment were CMT reagent, Ethanol (70%), and distilled water.